Safety of shrimp peptide concentrate as a novel food pursuant to Regulation (EU) 2015/2283

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Turck, Dominique; Bresson, Jean Louis; Burlingame, Barbara; Dean, Tara; Fairweather-Tait, Susan

Published in:
EFSA Journal

DOI:
10.2903/j.efsa.2018.5267

Publication date:
2018

SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 18 April 2018

doi: 10.2903/j.efsa.2018.5267

Safety of shrimp peptide concentrate as a novel food pursuant to Regulation (EU) 2015/2283

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), Dominique Turck, Jean-Louis Bresson, Barbara Burlingame, Tara Dean, Susan Fairweather-Tait, Marina Heinonen, Karen Ildico Hirsch-Ernst, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Monika Neuhäuser-Berthold, Grażyna Nowicka, Kristina Pentieva, Yolanda Sanz, Alfonso Siani, Anders Sjödin, Martin Stern, Daniel Tomé, Marco Vinceti, Peter Willatts, Karl-Heinz Engel, Rosangela Marchelli, Annette Pöting, Morten Poulsen, Josef Rudolf Schlatter, Leonard Matijević and Henk van Loveren

Abstract

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on shrimp peptide concentrate as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The NF is a peptide mixture obtained by an enzymatic proteolysis from northern shrimp (*Pandalus borealis*) shells and heads. The information provided on the composition, specifications, batch-to-batch variability, stability and production process of the NF is sufficient and does not raise safety concerns. The intention of the applicant is to use this NF as an ingredient in food supplements and to market it to adult consumers at a maximum proposed level of intake of 1,200 mg/day (corresponding to 17 mg/kg body weight (bw) per day for a 70 kg person). There are no concerns with regard to genotoxicity. The available human data do not raise safety concerns. Considering the no observed adverse effect level (NOAEL) of 2,000 mg/kg bw per day from a 90-day repeated-dose oral toxicity study, the maximum proposed level of intake and the nature of the NF, the Panel concludes that the margin of exposure (of 117) is sufficient. The Panel concludes that the NF, shrimp peptide concentrate, is safe to be used as a food supplement at the proposed maximum dose of 1,200 mg/day. The target population is adults. The Panel considered that the conclusion on the safety of the NF could not have been reached without the data from the unpublished study report on repeated-dose 90-day oral toxicity and from the unpublished study reports on two human studies.

Keywords: shrimps (*Pandalus borealis*), bioactive peptides, ACE-inhibitors, novel food, safety

Requestor: European Commission following an application by Medfiles Ltd (on behalf of the Marealis AS Company)

Question number: EFSA-Q-2017-00679

Correspondence: nda@efsa.europa.eu

Panel members: Dominique Turck, Jean-Louis Bresson, Barbara Burlingame, Tara Dean, Susan Fairweather-Tait, Marina Heinonen, Karen Ildico Hirsch-Ernst, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Monika Neuhäuser-Berthold, Grażyna Nowicka, Kristina Pentieva, Yolanda Sanz, Alfonso Siani, Anders Sjödin, Martin Stern, Daniel Tomé, Henk van Loveren, Marco Vinceti and Peter Willatts.

Suggested citation: EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), Turck D, Bresson J-L, Burlingame B, Dean T, Fairweather-Tait S, Heinonen M, Hirsch-Ernst KI, Mangelsdorf I, McArdle HJ, Naska A, Neuhäuser-Berthold M, Nowicka G, Pentieva K, Sanz Y, Siani A, Sjödin A, Stern M, Tomé D, Vinceti M, Willatts P, Engel K-H, Marchelli R, Pöting A, Poulsen M, Schlatter JR, Matijević L and van Loveren H, 2018. Scientific Opinion on the safety of shrimp peptide concentrate as a novel food pursuant to Regulation (EU) 2015/2283. EFSA Journal 2018;16(5):5267, 17 pp. https://doi.org/10.2903/j.efsa.2018.5267

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Summary

Following a request from the European Commission, the European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on shrimp peptide concentrate as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The assessment of the safety of this NF, which follows the methodology set out in the EFSA Guidance on the preparation and presentation of an application for authorisation of a novel food Regulation (EU) 2015/2283 (EFSA NDA Panel, 2016) and in the Commission Implementing Regulation (EU) 2017/2469, is based on the data supplied in the original application, the initial assessment by the competent authority of Finland, the concerns and objections of a scientific nature raised by the other Member States and information submitted by the applicant following an EFSA request for supplementary information and additional data identified by the Panel.

The 'Refined shrimp peptide concentrate' is a peptide mixture, obtained by enzymatic proteolysis from northern shrimp (*Pandalus borealis*) shells and heads. It contains more than 87% peptides (> 99.9% of peptides have molecular weight (MW) < 2 kDa), with less than 1% of both fat and carbohydrates. According to results of *de novo* sequencing, the NF contains more than 25,000 peptides in the range of 2–24 amino acids and 670 peptides were identified as being common for all tested batches. The information provided on the composition, the specifications, the batch-to-batch variability, stability and production process of the NF are sufficient and do not raise concerns about the safety of the NF.

The NF is proposed to be used only as an ingredient in food supplements, with the intention of the applicant to market it to consumers who want to control their blood pressure. The maximum proposed level of intake is 1,200 mg/day (corresponding to 17 mg/kg body weight (bw) per day for a 70 kg person). It can be assumed that a large portion of the peptides would be hydrolysed to the individual constituent amino acids prior to absorption and only a small fraction would be available for systemic uptake.

There are no concerns with regard to genotoxicity given the nature of the NF and on the basis of the studies provided by the applicant. The no observed adverse effect level (NOAEL) in a 90-day repeated-dose oral toxicity study was 2,000 mg/kg bw per day, the highest dose of the NF tested.

Human data included two clinical trials which, as primary endpoints, assessed potential effects of the NF on blood pressure in subjects with mild or moderate hypertension. These studies also assessed safety-related end points such as biometrics, clinical biochemistry and urine analysis parameters as well as recordings of adverse events. No statistically significant difference was observed between the treatment and placebo groups in regards to safety-related end points when the NF was taken in doses of 1,200 mg/day, over a treatment period of 8 weeks. The Panel considers that observed changes in blood pressure do not pose safety concerns in subjects with mild or moderate hypertension. Also, considering the nature of the NF and the exposure of humans to the large variety of proteins and peptides in the customary diet, as well as their fate in the intestine (hydrolysis), and because the changes on the blood pressure in mild and moderate hypertensive subjects were not of safety concern, the Panel considers that it is unlikely that the NF would have safety relevant effects in normo- or hypotensive subjects.

Taking into account the NOAEL of 2,000 mg/kg bw per day and maximum proposed level of intake of 17 mg/kg bw per day for adults, the Panel considers the resulting margin of exposure (117) to be sufficient.

The Panel concludes that the NF, shrimp peptide concentrate, is safe to be used as a food supplement at the proposed maximum dose of 1,200 mg/day. The target population is adults.

The Panel considered that the conclusion on the safety of the NF, shrimp peptide concentrate, could not have been reached without the data from the unpublished study report on repeated dose 90-day oral toxicity (2011c), and from the unpublished study reports on two human studies (2013, 2016).

Table of contents

1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

On 22 December 2016, the company Medfiles Ltd (on the behalf of the Marealis AS Company) submitted a request in accordance with Article 4 of the Novel Food regulation (EU) N° 258/97[1] to place on the market shrimp peptide concentrate as a novel food (ingredient) (NF).

On 8 March 2017, the competent authority of Finland forwarded to the Commission its initial assessment report, which came to the conclusion that shrimp peptide concentrate meets the criteria for acceptance of a NF defined in Article (3)1 of Regulation (EU) N° 258/97.

On 13 March 2017, the Commission forwarded the initial assessment report to the other Member States (MS). Several MSs raised objections or submitted comments.

The concerns of a scientific nature raised by the MSs can be summarised as follows:

- Safety concerns regarding a putative antihypertensive effect in (hypo-, normo-, and hypertensive) consumers. This should address blood pressure reduction as such, but also potential side effects related to the postulated mode of action, i.e. inhibition of the angiotensin converting enzyme (ACE) and cardiac effects. Possible interactions with medicines should also be assessed.

On 21 September and in accordance with Article 29(1)(a) of Regulation (EU) N° 178/2002[2], the Commission asked the European Food Safety Authority to provide a scientific opinion by carrying out the additional assessment for shrimp peptide concentrate as a NF in the context of Regulation (EU) N° 258/97 and to consider the elements of a scientific nature in the comments raised by the other MSs.

According to Article 35 (1) of Regulation (EU) 2015/2283[3], any request for placing a novel food on the market within the Union submitted to a Member State in accordance with Article 4 of Regulation (EU) N° 258/97, and for which the final decision has not been taken before 1 January 2018, shall be treated as an application under this Regulation. (Note: This is the case for this application).

In accordance with Article 10 (3) of Regulation (EU) 2015/2283, EFSA shall give its opinion as to whether the update of the Union List referred to in Article 10 (1) is liable to have an effect on human health.

2. Data and methodologies

2.1. Data

The safety assessment of this NF is based on data supplied in the original application, the initial assessment by the competent authority of Finland, the concerns and objections of a scientific nature raised by the other MSs and information submitted by the applicant in response to the MSs' comments and following an European Food Safety Authority (EFSA) request for supplementary information as well as additional data identified by the Panel.

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in the Commission Implementing Regulation (EU) 2017/2469[4].

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of a NF application (EFSA NDA Panel, 2016). As indicated in this guidance, it is the duty of the applicant to provide all of the available (proprietary, confidential and published) scientific data, including both data in favour and not in favour to support the safety of the proposed NF.

[1] Regulation (EU) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. *OJ L* 43, 14.2.1997, p. 1–6.

[2] Regulation (EU) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. *OJ L* 31, 1.2.2002, p. 1–24.

[3] Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001 (2013/0435 (COD). OJ L 327, 11.12.2015, p. 1–22.

[4] Commission Implementing Regulation (EU) 2017/2469 of 20 December 2017 laying down administrative and scientific requirements for applications referred to in Article 10 of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. *OJ L* 351, 30.12.2017, pp. 64-71.

This NF application includes a request for the protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283. Data claimed to be proprietary by the applicant include: Unpublished study report, undated; Analytical report of ACE-inhibitory effect of Marealis Refined Shrimp Peptide Concentrate; Unpublished study reports, 2009, 2011a–c, 2013, 2016.

2.2. Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications and the principles described in the relevant existing guidance documents from the EFSA Scientific Committee. The legal provisions for the assessment are laid down in Article 11 of Regulation (EU) 2015/2283 and in Article 7 of the Commission Implementing Regulation (EU) 2017/2469.

3. Assessment

3.1. Introduction

This assessment refers to an enzymatically hydrolysed shrimp peptide concentrate to be used as an ingredient in food supplements with the applicant's intention to market it to adult consumers who want to control their blood pressure.

This assessment concerns only risks that might be associated with the consumption of the NF under the proposed conditions of use and is not an assessment of the efficacy of the shrimp peptide concentrate with regard to any claimed benefit.

3.2. Identity of the NF

The NF named by the applicant 'Refined shrimp peptide concentrate' is a peptide mixture obtained by enzymatic hydrolysis of shrimp shells and heads.

3.3. Production process

The shrimp shells and heads are obtained from shrimps of the species *Pandalus borealis,* as a by-product of the production of cooked and peeled shrimps. The raw material is stored below 4°C and used by the applicant within 24 h after peeling.

The enzyme (protease) is obtained from non-pathogenic and non-toxicogenic *Bacillus licheniformis* and/or *Bacillus amyloliquefaciens* and complies with the recommended purity specifications for food enzymes given by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2006) and the Food Chemicals Codex (FCC, 2017). The applicant stated that this enzyme is an approved food enzyme in Denmark and France, and that it may be used as processing aid in other European Union (EU) countries, where no specific enzyme regulation applies at present.

At the start of the production of the NF, shrimp shells and heads, tap water and the food grade protease are mixed in a fixed ratio in a tank designated for hydrolysis. The hydrolysis follows a well-documented procedure as time and temperature are concerned. At the end of the hydrolytic process, the temperature is increased to a minimum of 90°C for at least 10 minutes to inactivate the enzyme (proteolytic) activity. The hydrolysate and the shrimp shells and heads are mechanically separated with the help of a sieve.

The hydrolysate, containing peptides, fat and minerals is then automatically and, with the help of pumps in a closed system, further processed in a decanter/tricanter to remove fat, fat bound components and larger molecules. Then, it is subjected to membrane filtration (ultrafiltration with a molecular weight (MW) cut-off of 1 kDa) to ensure that only peptides with a MW $<$ 2 kDa are present in the product. Water is then removed by reverse osmosis and evaporation before the hydrolysate is subjected to electrodialysis (ED) for the removal of NaCl. The de-salted hydrolysate is spray dried into a fine powder, ready for packaging in sealed plastic bags with an outer fibre drum.

Through all process steps, except for the ED, the temperature of the hydrolysate is kept $>$ 72°C to eliminate the growth of pathogenic bacteria.

The applicant gives further information about the residual enzymatic activity in the product. The protease is inactivated during the production process by heating, as demonstrated by measuring the protease activity in four samples of the NF by the method developed by Barret and Heath (1972).

The Panel noted that inactivation of an enzyme does not mean that the enzyme is removed. However, since the product undergoes a polymembrane cut-off of 1 kDa, it is envisaged that only small peptides to be present. It is also noted that 99.9% of peptides in NF have a MW < 2 kDa.

Regarding the quality control of the manufacturing process, the applicant has established two Critical Control Points (CCPs) at different steps during the process as part of their Hazard Analysis and Critical Control Point (HACCP) plan.

The Panel considers that the production process is sufficiently described and does not raise safety concerns.

3.4. Compositional data

The NF contains more than 87% peptides in the range of 2–24 amino acids, less than 1% both fat and carbohydrates, with minerals typical for shrimp shell and head material: sodium (< 35 mg/g), calcium (≤ 20 mg/g) and potassium (≤ 1.5 mg/g). The total dry matter of the NF is more than 95%.

The applicant provided results from seven production batches of the NF, which contained peptides with a MW < 2 kDa. The peptides separated by liquid chromatography (LC), were analysed using a Q-Exactive mass spectrometer. Data were collected in a data-dependent mode using a top10 method. *De novo* sequencing of peptides was performed using the PEAKS Studio 7 software, using an Average Local Confidence (ALC) score > 50% for identification. On the base of the results obtained, the applicant concluded that the NF contains more than 25,000 peptides in the range of 2–24 amino acids and that 670 peptides were identified as being common for all batches, (Unpublished study report, undated). The analytical method used is not accredited, but it is recognised by the scientific community (Zhang et al., 2012; Caron et al., 2016).

The applicant also provided the amino acid content of the NF and stated that it corresponds to that of the *P. borealis* shell and head fraction and that it has not been changed during the manufacturing process. The amino acid content of the NF was analysed in five batches by measuring the total amino acids (TAA) amount obtained by acidic hydrolysis followed by high-performance liquid chromatography (HPLC) analysis. Three amino acids, tryptophan, cysteine and methionine, were analysed separately (alkaline hydrolysis) since they get easily oxidised. The most abundant amino acid turned out to be glutamic acid (> 10 g/100 g), followed by aspartic acid and lysine.

To get more detailed information on the amino acid content of the NF, the applicant carried out additional analysis of free amino acids (FAA) in four different batches. These analyses show that both essential and non-essential FAA are present in the NF, with leucine as the most abundant.

The applicant provided chemical and microbiological analyses of five batches of the NF (results are reported in Table 1).

Table 1: Batch-to-batch analyses of the NF

Parameter (unit)	Product analyses for shrimp peptide concentrate				
	Batch No				
	710-2011-22370001	710-2011-22370002	710-2012-01684001	710-2013-22258001	710-2016-17982001
Identification and characterisation					
Total dry matter (%)	99.7	98.2	99.2	98.1	95.0
Peptides (%)	91.5	91.5	95.9	91.8	87.1
Ash (%)	12.3	10	8	8.6	8.5
Fat (g/100 g)	< 0.1	< 0.1	0.1	< 0.1	0.27
Carbohydrates (g/100 g)	0	0	0	< 0.2	< 0.2
Calcium (mg/kg)	14,000	12,000	3,100	13,000	16,000
Potassium (mg/kg)	1,100	300	460	450	430
Sodium (mg/kg)	32,000	25,000	24,000	17,000	18,900[(a),(b)]
Chemical purity					
Arsenic (inorganic) (mg/kg)	0.16	0.12	0.14	0.10	< 0.10
Arsenic (organic) (mg/kg)	42[(c)]	51	30[(c)]	46	27
Cadmium (mg/kg)	0.01[(d)]	0.01	0.02[(d)]	0.07	0.02

Parameter (unit)	Product analyses for shrimp peptide concentrate				
	Batch No				
	710-2011-22370001	710-2011-22370002	710-2012-01684001	710-2013-22258001	710-2016-17982001
Lead (mg/kg)	0.18[(c)]	0.11	0.16	< 0.05	< 0.05
Total Mercury (mg/kg)	0.028	0.024	0.009	< 0.005	0.008
Microbial purity					
Total viable count (CFU/g)	13,400[(e),(f)]	10,900[(e),(f)]	14,000	< 10,000	110[(g)]
Salmonella (/25 g)	ND[(e)]	ND[(e)]	ND	ND	ND[(g)]
Listeria sp. (/25 g)	ND[(e)]	ND[(e)]	ND	ND	ND
β-glucuronidase-positive *Escherichia coli* (CFU/g)	< 10[(e)]	< 10[(e)]	< 10	< 10	< 10
Coagulase positive *Staphylococcus aureus* (CFU/g)	< 100[(e)]	< 100[(e)]	< 100	< 100	< 10
Pseudomonas aeruginosa (/25 g)	ND	ND	ND	ND	< 10[(h)]
Yeast (and moulds) (CFU/g)	< 10	< 10	< 10	10	< 10[(i)]
Moulds (CFU/g)	< 10	< 10	< 10	10	< 10[(i)]

(a): Average value based on two samples.
(b): Analysed using ICP-SFM (Inductively Coupled Plasma-Supercritical Fluid Chromatography).
(c): Analysed using ISO 11885, mod., ICP-OES (Inductively Coupled Plasma-Optical Emission Spectroscopy).
(d): Analysed using §64 LFGB L00.00-19/3, AAS-Gr (Atomic Absorption Spectroscopy-Graphite Furnace).
(e): Analysed by Tos Lab.
(f): Analysed using ISO 4833-1.
(g): Analysed using ISO 6579-1.
(h): Analysed using ISO 13720.
(i): Analysed using §64 LFGB L01.00-37.

The Finnish Food Safety Authority (Evira), as initial assessor, requested further information on the astaxanthin content of the product, because shrimps are a natural source of this carotenoid, which gives them their reddish colour. The applicant provided analyses made for three product batches, in which the astaxanthin content, both free astaxanthin and astaxanthin esters, was < 1 g/100 g and < 3 mg/kg, respectively, and for all three batches under the detection limit of the method used. Noting that astaxanthin is fat soluble and given the limit for fat content (< 1%) in the batch-to-batch analyses reported in Table 1, the Panel considers that the potential content of astaxanthin in the NF would be low and of no concern.

The levels of heavy metals (lead, mercury and cadmium) in the NF (Table 1) are below the maximum levels in food supplements given in the Commission Regulation (EU) No 1881/2006[5]. Regarding arsenic (organic and inorganic), there are no maximum levels set for food supplements, only for inorganic arsenic in rice and rice products set in Commission Regulation (EU) No 2015/1006[6]. The applicant compared the analytical values for inorganic arsenic in the NF with the maximum levels set for rice and noted that they are below these levels (0.20–0.30 mg/kg) and close to the maximum levels set for rice for infants and young children (0.10 mg/kg).

A MS requested additional analysis for organic mercury (methylmercury) in NF. The applicant replied providing results of additional analysis for four new batches, which were: 0.027 mg/kg, 0.020 mg/kg, < 0.010 mg/kg and < 0.010 mg/kg, respectively.

Regarding levels of environmental contaminants, the applicant provided analytical results for dioxins and polychlorinated biphenyls (PCBs). Currently, there are no maximum levels in EU legislation for these contaminants set for shrimp shells and heads or food supplements, so the applicant compared analytical results with maximum levels for muscle meat of fish and fishery products and products thereof given in Commission Regulation No 1881/2006 and noted that the amounts are well below.

The Panel considers that the information provided on the composition of the NF is sufficient and does not raise safety concerns.

[5] Commission Regulation (EC) 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.
[6] Commission Regulation (EU) 2015/1006 of 25 June 2015 amending Regulation (EC) No 1881/2006 as regards maximum levels of inorganic arsenic in foodstuffs.

3.4.1. Stability

The applicant examined the stability of the NF (in powder form) by measuring the ACE-inhibitory activity over 24 months at room temperature (20°C) and higher temperature (40°C) under dry conditions. The ACE-inhibition for one batch was analysed every 1, 3, 6, 12, 18 and 24 months with the method suggested by Vermeirssen et al. (2002). The applicant concluded that there was no significant difference in the ACE-inhibitory effect of the NF throughout the storage period, regardless of the temperature (Gildberg et al., 2011).

The stability of the NF was also assessed by evaluating the dry matter content, the water activity, colour and odour of three batches up to 8 years. The results of the analyses of the NF for these parameters indicate that the ingredient is stable up to 8 years, with minimal risk of microbial growth.

In addition, the presence of microorganisms was tested in the food supplements containing the NF, (i.e. tablets manufactured for the first pilot clinical trial), during the storage at room temperature (up to 25°C) and protected from humidity. The analysis was made using accredited methods except that for moulds. The data provided indicate that the tablets containing the NF are stable from the microbiological point of view up to 6 years of storage.

Considering the composition and the intended use levels of the NF and provided that NF is appropriately packaged and stored, the Panel has no safety concerns regarding the stability of the NF.

3.5. Specifications

Detailed product specifications, provided by the applicant are reported in Table 2.

Table 2: Specifications of the NF proposed by the applicant

Parameter	Specification (Unit)	Method
Identification and characterisation		
Total dry matter	> 95 (%)	Gravimetry
Peptides	> 87 (%)	Titrimetry (N × 6.25) Kjeldahl
Peptides with molecular weight < 2 kDa	> 99.9 (%)	LC-MS/MS (MALDI TOF and Q-TOF)
Fat	< 1.0 (g/100 g)	Gravimetry (Weibull)
Carbohydrates	< 1.0 (g/100 g)	Calculated (balance method)
Ash	< 15 (%)	Gravimetry
Calcium (Ca)	≤ 20,000 (mg/kg)	ICP-OES
Potassium (K)	≤ 1,500 (mg/kg)	ICP-OES
Sodium (Na)	< 35,000 (mg/kg)	ICP-OES
Chemical purity		
Arsenic (As)		
Inorganic	< 0.22 (mg/kg)	§64 LFGB 25.06, HG-AAS
Organic	< 51 (mg/kg)	EN 15763:2009, ICP-MS
Cadmium (Cd)	< 0.09 (mg/kg)	EN 15763:2009, ICP-MS
Lead (Pb)	< 0.18 (mg/kg)	EN 15763:2009, ICP-MS
Total mercury (Hg)	< 0.03 (mg/kg)	§64 LFGB L00.00-19/4, CV-AAS
Microbial Purity		
Total viable count	≤ 20,000 (CFU/g)	§64 LFGB L00.00-88
Salmonella	ND (/25 g)	§64 LFGB L00.00-20[(a)]
Listeria monocytogenes	ND (/25 g)	ISO 11290-1[(a)]
Escherichia coli	< 20 (CFU/g)	ISO 16649-2[(a)]
Coagulase positive *Staphylococcus aureus*	< 200 (CFU/g)	ISO 6888-1[(a)]
Pseudomonas aeruginosa	ND (/25 g)	Ph. Eur. 2.6.13
Yeast and moulds	< 20 (CFU/g)	Ph. Eur. 2.6.12

CV-AAS: cold vapour/atomic absorption spectroscopy; CFU: colony forming units; HG-AAS: hydride generation-atomic absorption spectroscopy; ICP-MS: inductively coupled plasma mass spectrometry; ICP-OES: inductively coupled plasma- optical emission spectrometry; MALDI TOF: matrix-assisted desorption ionisation-time of flight; Q-TOF: quadrupole-time of flight mass spectrometry; LFGB: German food and feed code; Ph. Eur.: European Pharmacopoeia; ND: not detected.
(a): Horizontal method.

The Panel noted that some analytical methods reported in Table 1 were different from those listed in Table 2. Upon the Panel's request for an explanation, the applicant responded that, on account of the intervening wide time period, some analyses were made in different laboratories, using different methods.

The results of the analysis of the MW of peptides were obtained on additional five batches using matrix-assisted desorption ionisation-time of flight (MALDI TOF) and quadrupole-time of flight mass spectrometry (Q-TOF) mass spectrometers and were in compliance with the specification for this parameter (> 99.9% of peptides have MW < 2 kDa).

The applicant demonstrated the microbiological quality of the NF by the analysis of five different batches for a total viable count of bacteria, *Salmonella*, *Listeria* sp., ß-glucuronidase positive *Escherichia coli*, coagulase positive *Staphylococcus aureus, Pseudomonas aeruginosa,* yeast and moulds.

The analysis was made using accredited methods, except for the method for mould analysis, which however, is generally used. Based on the analytical results, the microbiological quality of the NF complies with the specification.

The Panel considers that the information provided on the specification and the batch-to-batch variability of the NF is sufficient and does not raise safety concerns.

3.6. History of use of the NF and/or of its source

3.6.1. History of use of the source

The NF is manufactured from shells and heads of northern shrimps (*P. borealis*).

Northern shrimps are sold in the EU frozen either as a whole with shells and heads or peeled. They have been traditionally used in the European diet, mostly peeled before consumption.

There is no information about the intake of shrimp shells or heads as food in the European diet.

3.6.2. History of use of the NF

The applicant provided information that, on the 13 April 2017, the NF was approved in Canada by Health Canada for the same proposed use as in the application submitted to EFSA. However, the applicant did not provide data on the actual consumption of the NF.

3.7. Proposed uses and use levels and anticipated intake

3.7.1. Target population

The applicant's intention is to market the NF to consumers who want to control their blood pressure. For this assessment, EFSA considers that the target population is the general adult population.

3.7.2. Proposed uses and use levels

The applicant intends to use NF only as an ingredient of food supplement applications, e.g. tablets, capsules, powder, etc. and not as an ingredient in any other food products.

The maximum daily dose proposed by the applicant is 1,200 mg of NF (which corresponds to 17 mg/kg body weight (bw) per day for adults[7]), e.g. two tablets per day, each tablet containing 600 mg of NF.

3.7.3. Precautions and restrictions of use

The applicant indicated that the NF is not intended for consumption by children or as an alternative to or in combination with blood pressure lowering medicines for the treatment of hypertensive subjects.

3.8. Absorption, distribution, metabolism and excretion (ADME)

Considering the source and nature of the NF, it can be assumed that a large portion of the peptides would be hydrolysed to the individual constituent amino acids prior to absorption and only a small fraction would be available for systemic uptake. Following systemic absorption, peptides are subject to hydrolysis in blood or other organ tissues, including the liver and kidneys. No information has been provided on the absorption of FAA, di- and tripeptides.

[7] By using a mean bw of 70 kg for adults (EFSA Scientific Committee, 2012)

3.9. Nutritional information

To compare the amino acid profiles of the NF with shrimp meat, the applicant provided the results of analyses of the amino acid content of the NF (mean value of five different batches) and of shrimp meat. The amino acid profiles of the NF and meat are similar. The Panel noted that the analysis on shrimp meat was done on only one sample.

At the maximum daily dose of the NF proposed by the applicant (i.e. 1,200 mg in food supplements), the intake of peptides would be around 1 g, and the contents of fat and carbohydrates in the NF are both below 1%. The Panel considers that consumption of the NF is not nutritionally disadvantageous.

3.10. Toxicological information

The Panel notes that the toxicological studies, which are discussed in the following sections (Sections 3.10.1 and 3.10.2), were performed using an intermediate product of the manufacturing process as test material, instead of the NF.

The applicant declared that the intermediate product was obtained before the ultrafiltration step had been implemented in the production process. It differs from the NF mainly as far as the peptide size (MW) is concerned. Namely, the NF contains mainly peptides with MW < 2 kDa, whereas the intermediate product contains also small amounts of larger peptides. The comparison of the amounts of peptides with different sizes in the intermediate product and in the NF is shown in Table 3.

Table 3: Characterisation of the intermediate product and the NF

Parameter	Intermediate product	NF
Peptides	89.5%	> 87%
Peptides with molecular weight < 2 kDa	95.8%	> 99.9%
Peptides with molecular weight 2–4 kDa	3.5%	< 0.1%
Peptides with molecular weight 4–6 kDa	0.5%	0%
Peptides with molecular weight 6–8 kDa	0.1%	0%
Peptides with molecular weight > 8 kDa	~ 0.1%	0%

Despite the small differences, i.e. the intermediate product contains also a 4% fraction with peptides above 2 kDa, the Panel considers that the use of this preparation is appropriate for the toxicological testing.

3.10.1. Genotoxicity

The applicant provided a bacterial reverse mutation test using the intermediate product as test material (unpublished study report, 2011a). The study was performed in compliance with the OECD Principles of good laboratory practice (GLP) (OECD, 1998a) and according to OECD Test Guideline No. 471 (OECD, 1997a).

In a preliminary test using *Salmonella Typhimurium* tester strain TA100 and the plate incorporation method, the intermediate product did not show cytotoxicity in the absence and presence of a metabolic activation system (S9 mix) up to a dose of 5,000 μg/plate. The main test, using *S. Typhimurium* tester strains TA1537, TA1535, TA98, TA100 and TA102 and the plate incorporation method, comprised two independent experiments. Both in the absence and presence of a metabolic activation system, the test material did not induce a biologically relevant increase in revertant colony numbers compared with the respective negative control (tester strains treated with distilled water) in any of the five strains up to the highest tested dose of 5,000 μg/plate. The intermediate product was thus not mutagenic in this study.

The applicant also provided a mammalian bone marrow chromosome aberration test in mice using the intermediate product as test material (unpublished study report, 2011b). The study was performed in compliance with the OECD Principles of GLP (OECD, 1998a) and according to the OECD Test Guideline No. 475 (OECD, 1997b).

A dose-range finding study was performed to determine whether the test material produces toxic effects in Swiss albino mice. Two animals per sex per group were administered the intermediate product at dose levels of 0 (vehicle control), 250, 500, 1,000 or 2,000 mg/kg bw by oral gavage for

two consecutive days. All animals appeared normal before euthanisation. Bone marrow samples were collected and the toxicity to bone marrow cells was assessed by the determination of the percent mitotic index. A dose-related reduction in the percent mitotic index was observed, which reached 36% in the male animals and 31% in the female animals at the highest dose level. Based on these results, the main study was conducted as a limit test at a dose of 2,000 mg/kg bw administered for two consecutive days.

In the main study, groups of 5 male and 5 female animals were administered the intermediate product by gavage at a dose of 2,000 mg/kg bw on two consecutive days. The negative control group received the vehicle (saline 0.9% w/v) by oral gavage, and the positive control group received a single intraperitoneal injection of mitomycin C at a dose of 4 mg/kg bw on the second day. All animals appeared normal until they were euthanised 1 day after the final treatment (i.e. after approximately 1.5 normal cell cycle length). Approximately 3 h prior to sacrifice, all animals were injected intraperitoneally with colchicine (4 mg/kg bw) to arrest the cells in metaphase. Bone marrow cells were obtained from femurs, and metaphase cells were analysed for chromosome aberrations.

As in the dose range finding study, a statistically significant reduction in the percent mitotic index was observed in male and female animals treated with the intermediate product (23.4 and 23.0%, respectively) when compared with the negative control group. There was no statistically significant increase in the percentage of cells with structural chromosome aberrations (excluding gaps) in animals treated with the test material when compared with the vehicle control group. A reduction in the percent mitotic index and a significant increase in the percentage of cells with structural chromosome aberrations in the animals treated with the positive control mitomycin C demonstrated the sensitivity of the test system. The Panel concludes that the intermediate product was not clastogenic after administration to Swiss albino mice at a dose of 2,000 mg/kg bw per day for two consecutive days.

Even though an *in vitro* micronucleus test, as recommended in the EFSA Scientific Opinion on genotoxicity testing strategies (EFSA Scientific Committee, 2011), was not conducted, the Panel considers that given the nature of the NF and the results of the studies presented, there are no concerns with regard to genotoxicity.

3.10.2. Acute and subchronic toxicity

The applicant provided an acute oral toxicity study using the intermediate product of the manufacturing process as test material (unpublished study report, 2009). The study was conducted in compliance with the OECD Principles of GLP (OECD, 1998a) and in accordance with OECD Test Guideline No. 423 (OECD, 2001). After administration of the test material at a single dose of 2,000 and 5,000 mg/kg bw, respectively, to groups of female Wistar rats (n = 3), no adverse effects were observed.

The intermediate product was tested in a repeated-dose 90-day oral toxicity study using rats (unpublished study report, 2011c). The study was performed following the OECD Principles of GLP (OECD, 1998a) and according to OECD Test Guideline No. 408 (OECD, 1998b).

Groups of 10 male and 10 female Wistar rats were administered the test material by gavage at dose levels of 0 (vehicle control: saline 0.9% w/v), 100, 500 or 2,000 mg/kg bw per day for 90 consecutive days. Feed and water were provided ad libitum. During the treatment period, the animals were checked twice daily for mortality and clinical signs. Detailed clinical examinations/ neurobehavioural observations were conducted on each animal once prior to initiation of treatment and weekly thereafter. Ophthalmoscopy was performed pretreatment and prior to terminal sacrifice. Functional observational battery (FOB) and motor activity tests were conducted during the 12th week of treatment. Blood samples for haematological and clinical chemistry analysis, as well as urine samples for urinalysis, were taken from all animals at the end of the treatment period. All rats were sacrificed and subjected to a detailed necropsy examination with selected organs weighted. Organs and tissues of all animals were collected and preserved, and those of the animals in the high-dose and the control group were subjected to a comprehensive histopathological examination.

No mortality was observed during the treatment period. Daily observations, as well as weekly detailed clinical observations of the animals, did not identify clinically relevant signs attributable to treatment with the test material. FOB and motor activity evaluations revealed no treatment-related changes; isolated statistically significant differences between the test groups and the control group can be considered as incidental findings. Ophthalmoscopic examinations did not identify any abnormality.

Body weights and feed consumption in the test groups showed no statistically significant differences compared with the control group. Significantly lower body weight gains in males of the

low- and mid-dose groups during week 2 are not regarded as treatment-related by the Panel since the differences were transient and did not correlate with the administered dose.

Haematological analysis at the end of the treatment period showed statistically significantly higher plasma haemoglobin (Hb) level in females of the mid- and high-dose groups when compared with the control group. A slightly non-significantly higher mean value was also noted for females of the low-dose group. The differences to the controls were small (< 5%), and the mean values fell well within the range of the historical control means, whereas the mean value for the control group was at the lower end. Therefore, the Panel considers the observed differences in Hb levels as not adverse. Furthermore, in the absence of a clear relation to dose, they are probably unrelated to treatment with the test material. Significantly higher mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) in females (all three dose groups) and MCHC in males (mid- and high-dose groups) resulted from the slightly higher mean Hb levels in these groups. Since the differences compared with the control group were small (≤ 5% in the high-dose group) and the mean values fell well within the range of the historical control means, whereas the mean value for the control group was at the lower end, these findings were also not considered adverse. Significantly higher white blood cell counts (WBC) in females of the low- and mid-dose groups are not considered treatment-related in the absence of a relation to dose.

Clinical chemistry analysis identified statistically significantly increased levels of blood urea nitrogen (BUN) and urea in males of the high-dose group (12% and 23%, respectively) when compared with the control group. Females of the mid- and high-dose groups showed a (non-significant) trend towards higher BUN and urea levels. The Panel considers that these effects were treatment-related metabolic changes caused by the administration of the peptide-rich test material, as has been shown for other protein-rich diets (Kleinknecht et al., 1979; Chevalier et al., 2010; Kapurkar, 2012, 2014). Globulin levels were significantly increased only in males of the mid- and high-dose groups, resulting in significantly higher total protein levels and lower albumin/globulin ratios. The lack of a dose-response suggests that the effects are not treatment-related. In the absence of changes in other parameters, gross and histopathological findings, the Panel considers that the increased globulin levels, observed in males only, are not toxicologically relevant.

Females of all three dose groups showed significantly higher sodium and chloride levels. Regarding both parameters, the differences to the controls were minimal (≤ 2.6%), and not considered adverse. Furthermore, the absence of a relation to the administered dose suggests that the observed higher sodium and chloride levels are not treatment-related. This also applies to a significantly lower potassium level in females of the mid-dose group and a significantly lower creatinine level in males of the mid-dose group.

Urinalysis revealed a statistically significantly lower specific gravity in males of the high-dose group when compared with the control group. This minimal change (1.1%) is regarded as not biologically relevant by the Panel. A significantly higher pH was observed in males of all three dose groups (dose-related). Considering that the mean pH for the control group was lower than the historical control means and that there were no changes in any parameter indicating kidney toxicity, these variations in urine pH observed in males, only, do not raise safety concerns.

Organ weight determinations showed no statistically significant differences in the test groups compared with the control group, except for a significantly higher relative kidney weight in males of the low-dose group and a lower relative adrenal weight in females of the mid-dose group. These findings are not considered treatment-related since there was no correlation with the administered dose. Macroscopic examinations at necropsy revealed no gross pathological findings. Microscopic examinations of selected organs and tissues identified no treatment-related differences in the incidences of the histopathological findings between groups.

The Panel concludes that the intermediate product did not induce toxicity in Wistar rats after oral administration for 90 days. Thus, the no observed adverse effect level (NOAEL) was 2,000 mg/kg bw per day, the highest dose tested.

3.10.3. Chronic toxicity and carcinogenicity

No studies on chronic toxicity and carcinogenicity were provided.

3.10.4. Reproductive and developmental toxicity

No studies on reproductive and developmental toxicity were provided.

3.10.5. Human data

The applicant provided study reports on two clinical trials which, as primary endpoints, assessed potential effects of the NF on blood pressure in subjects with mild or moderate hypertension. These studies also assessed safety-related end points such as clinical biochemistry and urine analysis parameters as well as recordings of adverse events (AEs) (Unpublished study reports, 2013, 2016).

In a randomised, double-blind, placebo-controlled trial (RCT), 68 adult subjects between 30 and 75 years of age and with mild or moderate hypertension, but overall healthy, received the NF at dose levels of 0 (control) or 1,200 mg/day, for 8 weeks (unpublished study report, 2013). The primary objective was to assess the effect of the NF on blood pressure by observing a change in systolic blood pressure (SBP), during the 8-week intervention. The safety objectives included a number of clinical biochemistry parameters and the number and type of AEs. There were no significant differences in safety parameters between treatment and control groups.

In the second study, a multicentre RCT, adults between 30 and 75 years of age (138 of 144 completed the study; 126 were included in per-protocol analysis), with mild to moderate hypertension, but overall healthy, received 1,200 mg/day of the NF or placebo for 8 weeks (unpublished study report, 2016). The primary end points were to assess the change in daytime ambulatory SBP and the change in office SBP between the treatment group and the control group, during the 8-week intervention. Safety end points included recordings of biometrics, clinical biochemistry, and AEs. No statistically significant differences were found between groups in regards to the safety parameters evaluated.

The Panel considers that changes in blood pressure observed in these two clinical trials do not pose safety concerns in subjects with mild or moderate hypertension.

Other concerns expressed by MSs regarded the safety of the NF in normo- and hypotensive subjects. The Panel noted that there were no studies on the use of the NF in these subjects. Considering the nature of the NF and the exposure of humans to the large variety of proteins and peptides in the customary diet, as well as their fate in the intestine (hydrolysis), and because the changes in the blood pressure in mild and moderate hypertensive subjects were not of safety concern, the Panel considers that it is unlikely that the NF would have safety relevant effects in normo- or hypotensive subjects.

The Panel concludes that the available human data do not raise safety concerns.

3.11. Allergenicity

The NF is derived from shrimps which belong to crustaceans, a well-known allergenic food.

4. Discussion

The NF is a peptide mixture obtained from shrimp shells and heads via enzymatic hydrolysis. The proposed maximum daily intake of the NF is 17 mg/kg bw.

The information provided on composition, specifications, production process and stability of the NF, do not raise safety concerns.

There are no concerns with regard to genotoxicity. The NOAEL in a repeated-dose 90-day oral toxicity study on rats was 2,000 mg/kg bw per day, the highest dose tested.

In addition, two clinical studies on subjects with mild or moderate hypertension did not show changes in clinical biochemistry, biometrics and urinalysis parameters or adverse effects of a daily dose of 1,200 mg of NF, administered for 8 weeks.

Taken together, including the nature of the product, the Panel considers the resulting margin of exposure (117) (i.e. the ratio between the NOAEL and the proposed maximum daily intake) to be sufficient.

5. Conclusions

The Panel concludes that the NF, shrimp peptide concentrate, is safe to be used as a food supplement at the proposed maximum dose of 1,200 mg/day. The target population is adults.

The Panel considered that the conclusion on the safety of the NF, shrimp peptide concentrate, could not have been reached without the data from the unpublished study report on repeated-dose 90-day oral toxicity (2011c) and from the unpublished study reports on two human studies (2013, 2016).

Steps taken by EFSA

1) Letter from the European Commission to the European Food Safety Authority with the request for a scientific opinion on the safety of shrimp peptide concentrate. Letter SANTE/E2/TD/ln ARES (2017) 4350321, dated 06 September 2017.
2) On 21 September 2017, EFSA received a valid application from the European Commission on shrimp peptide concentrate as NF, which was submitted by Medfiles Ltd (on behalf of the Marealis AS Company), and the scientific evaluation procedure started.
3) On 06 November 2017, EFSA sent a request to the applicant to provide the missing information to accompany the application.
4) On 13 December 2017, EFSA received the missing information as submitted by the applicant. After checking the content of the full dossier, including the missing information, EFSA considered the application valid as of 19 December 2017.
5) On 24 January 2018, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
6) On 12 March 2018, additional information was provided by the applicant and the scientific evaluation was restarted.
7) During its meeting on 18 April 2018, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of shrimp peptide concentrate as a NF pursuant to Regulation (EU) 2015/2283.

References

Barret AJ and Heath MF, 1972. Lysosomal enzymes. In: Dingle JT (ed). *Lysozymes – A Laboratory Handbook*. North-Holland Publishing Company, Amsterdam. pp. 46–135.

Caron J, Chataign′e G and Gimeno J-P, 2016. Food peptidomics of in vitro gastrointestinal digestions of partially purified bovine hemoglobin: low-resolution versus high-resolution LC-MS/MS analyses. Electrophoresis, 2016 (37), 1814–1822. https://doi.org/10.1002/elps.201500559

Chevalier L, Bos C, Azzout-Marniche D, Dardevet D, Tomé D and Gaudichon C, 2010. Dietary protein regulates hepatic constitutive protein anabolism in rats in a dose-dependent manner and independently of energy nutrient composition. American Journal of Physiology: Regulatory, Integrative and Comparative Physiology, 299, R1720-30. https://doi.org/10.1152/ajpregu.00497.2010

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), Turck D, Bresson J-L, Burlingame B, Dean T, Fairweather-Tait S, Heinonen M, Hirsch-Ernst KI, Mangelsdorf I, McArdle H, Naska A, Neuhäuser-Berthold M, Nowicka G, Pentieva K, Sanz Y, Siani A, Sjödin A, Stern M, Tomé D, Vinceti M, Willatts P, Engel K-H, Marchelli R, Pöting A, Poulsen M, Salminen S, Schlatter J, Arcella D, Gelbmann W, de Sesmaisons-Lecarré A, Verhagen H and van Loveren H, 2016. Guidance on the preparation and presentation of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283. EFSA Journal 2016;14(11):4594, 24 pp. https://doi.org/10.2903/j.efsa.2016.4594

EFSA Scientific Committee, 2011. Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011;9(9):2379, 69 pp. https://doi.org/10.2903/j.efsa.2011.2379

EFSA Scientific Committee, 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp. https://doi.org/10.2903/j.efsa.2012.2579

FCC (Food Chemical Codex), 2017. Food Chemicals Codex 10th edition, 2016-2017, Supplement II. United States Pharmacopeial Convention (USP), Rockville, MD.

Gildberg A, Arnesen JA, Sæther B-S, Rauø J and Stenberg E, 2011. Angiotensin I-converting enzyme inhibitory activity in a hydrolysate of proteins from Northern shrimp (Pandalus borealis) and identification of two novel inhibitory tri-peptides. Process Biochemistry, 46, 2205–2209. https://doi.org/10.1016/j.procbio.2011.08.003

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2006. General specifications and considerations for enzyme preparations used in food processing. FAO JECFA Monographs 3, Annex 5: 89-95

Kapurkar U, 2012. Historical control data on numeric urinanalysis parameters in Wistar rats from 90 day toxicity studies (Under BMR Facilities). Pathology Section, Department of Toxicology, JAI Research Foundation, India. 3 pp.

Kapurkar U, 2014. Historical control data on various hematology, coagulation and clinical chemistry parameters in Wistar rats from 90 day toxicity studies (Under BMR Facilities). Pathology Section, Department of Toxicology, JAI Research Foundation, India. 6 pp.

Kleinknecht C, Salusky I, Breyer M and Gubler M-C, 1979. Effect of various protein diets on growth, renal function, and survival of uremic rats. Kidney International, 15, 534–541.

OECD (Organisation for Economic Co-operation and Development), 1997a. Test No. 471: Bacterial Reverse Mutation Test. In: OECD guideline for testing of chemicals, Section 4: Health effects, 11 pp.

OECD (Organisation for Economic Co-operation and Development), 1997b. Test No. 475: Mammalian Bone Marrow Chromosome Aberration Test. In: OECD guideline for testing of chemicals, Section 4: Health effects, 8 pp.
OECD (Organisation for Economic Co-operation and Development), 1998a. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring.
OECD (Organisation for Economic Cooperation and Development), 1998b. Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents, 10 pp.
OECD (Organisation for Economic Cooperation and Development), 2001. Test No. 423: Acute Oral Toxicity – Acute Toxic Class Method, 14 pp.
Unpublished study report, 2009. Acute oral toxicity study of Marealis BPC in rats. (Study No.: AOT-03/09)
Unpublished study report, 2011a. Bacterial reverse mutation test of Marealis BPC using *Salmonella typhimurium*. (JRF Study Number: 481-1-06-1445, Final report).
Unpublished study report, 2011b. Mammalian bone marrow chromosome aberration test of Marealis BPC in mice. (JRF Study Number: 484-1-06-1446, Final report).
Unpublished study report, 2011c. Repeated dose 90-day oral toxicity study of Marealis BPC in wistar rats. (JRF Study Number: 443-1-03-1400, Final report).
Unpublished study report, 2013. Assessment on Antihypertensive Effect and Safety of Bioactive Peptides Derived from Coldwater Shrimp (Pandalus borealis) in Healthy Subjects with Mild or Moderate Hypertension. (Clinical Study Report. Final). Oy Foodfiles Ltd, Kuopio Finland.
Unpublished study report, 2016. A Randomized, Double-blind, Placebo-controlled, Parallel Study for the Assessment of Anti-Hypertensive Effect and Safety of a Dietary Supplement, Marealis RPC (Refined Peptide Concentrate), in Healthy Subjects with Mild or Moderate Hypertension: Final Study Report. (Study Identification Number: 13TBHM). Prepared by London (ON): KGK Synergize Inc. for Tromsø, Norway: Marealis AS.
Unpublished study report, undated. *De novo* peptide sequences of Refined Shrimp Peptide Concentrate.
Vermeirssen V, Van Camp J and Verstraete W, 2002. Optimisation and validation of an angiotensin converting enzyme inhibition assay for screening of bioactive peptides. Journal of Biochemical and Biophysical Methods, 51, 75–87. https://doi.org/10.1016/S0165-022X(02)00006-4
Zhang J, Xin L, Shan B, Chen W, Xie M, Yuen D, Zhang W, Zhang Z, Lajoie GA and Ma B, 2012. 2012: PEAKS DB: de novo sequencing assisted database search for sensitive and accurate peptide identification. Molecular & Cellular Proteomics, 11, 1–8. https://doi.org/10.1074/mcp.M111.010587

Abbreviations

AAS-Gr	atomic absorption spectroscopy-graphite furnace
ACE	angiotensin converting enzyme
ADME	absorption, distribution, metabolism and excretion
AE	adverse events
ALC	average local confidence
BUN	blood urea nitrogen
bw	body weight
CCP	Critical Control Point
CFU	colony forming unit
CV-AAS	cold vapour/atomic absorption spectroscopy
ED	electrodialysis
FAA	free amino acids
FCC	Food Chemicals Codex
FOB	functional observational battery
GLP	Good Laboratory Practice
HACCP	Hazard Analysis and Critical Control Point
HG-AAS	hydride generation-atomic absorption spectroscopy
HPLC	high performance liquid chromatography
ICP-MS	inductively coupled plasma mass spectrometry
ICP-OES	inductively coupled plasma-optical emission spectroscopy
ISO	International Organization for Standardization
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC	liquid chromatography
LC-MS/MS	Liquid chromatography with tandem spectroscopy
LFGB	German Food and Feed Code
MALDI TOF	matrix-assisted desorption ionisation – time of flight
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration

MS	Member State
MW	molecular weight
ND	not detected
NDA	EFSA Panel on Dietetic Products, Nutrition and Allergies
NF	novel food
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
PCB	polychlorinated biphenyls
Ph. Eur.	European Pharmacopoeia
Q-TOF	quadrupole – time of flight mass spectrometry
RCT	randomised controlled trial
SBP	systolic blood pressure
TAA	total amino acids
WBC	white blood cell counts